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## TECHNICAL NOTE

SHELL VALVE MOVEMENT RESPONSE OF DARK FALSE MUSSEL, *MYTILOPSIS LEUCOPHAETA*, TO CHLORINATIONSANJEEVI RAJAGOPAL<sup>1\*</sup>, GERARD VAN DER VELDE<sup>1</sup> and HENK A. JENNER<sup>2</sup><sup>1</sup>Department of Ecology, Laboratory of Aquatic Ecology, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands; and <sup>2</sup>Joint Laboratories and Consulting Services of the Dutch Electricity Supply Companies (KEMA), P.O. Box 9035, 6800 ET Arnhem, The Netherlands

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**Abstract**—Shell valve movements of fouling mussel, *Mytilopsis leucophaeta*, have been studied in the presence of chlorine, using a mussel monitor. Data showed increasing shell valve closure with increasing chlorine concentration. Shell opening rates of *M. leucophaeta* at control experiments (0 mg litre<sup>-1</sup> residual chlorine) were about 10 times more than those at 1 mg litre<sup>-1</sup> residual chlorine. Continuous dosing of 0.75 mg litre<sup>-1</sup> residual chlorine is required before shell movements are critically affected. Since current environmental stipulations do not permit this, a level of 0.5 mg litre<sup>-1</sup> has to be used continuously during settlement periods of *M. leucophaeta* for their control. The results also indicate that *M. leucophaeta* is more tolerant to chlorine than other mussel species. © 1997 Elsevier Science Ltd

**Key words**—power station, bivalves, *M. leucophaeta*, chlorination, shell valve movement

## INTRODUCTION

Settlement and growth of fouling organisms in the cooling conduits of power stations is often a problem of considerable economic significance (Fischer *et al.*, 1984). Of all the organisms that constitute fouling in cooling systems, mussels are known to cause the most serious problems (Rajagopal *et al.*, 1996). Earlier studies at various power stations indicated that marine mussel, *Mytilus edulis* L., and fresh water mussel, *Dreissena polymorpha* (Pallas), were the most problematic mussel species affecting the normal operation of the power stations in The Netherlands (Jenner and Janssen-Mommen, 1993) and elsewhere (Jensen, 1982; Fischer *et al.*, 1984). The brackish water mussel, *Mytilopsis leucophaeta* (Conrad) (syn. *Congeria cochleata* Kickx in Nyst), is the dominant macrofouling species at the power stations operating in the Noordzeekanaal connecting Amsterdam harbours and the coast (Rajagopal *et al.*, 1994). Settlement densities as high as 6.5 million m<sup>-2</sup> have been reported near power-station intakes (Rajagopal *et al.*, 1995). This species was reported to be originated from the Atlantic coast of the United States and the Gulf of Mexico (Marelli and Gray, 1983).

Chlorination of cooling water, using chlorine gas or hypochlorite from electrolytic sources, has been reported to be one of the most effective methods of mussel control in industrial cooling systems (Lewis, 1985; Rajagopal *et al.*, 1996). However, increasing environmental concern about the discharge of chlorinated water to the environment has resulted in effluent stipulations being made stricter and stricter (Jenner, 1985). A study on response of *M. leucophaeta* to chlorine has great importance for the selection and optimization of chlorine treatments in the cooling systems. Therefore, the present study was organized to find out the behavioural response (shell valve movement) of *M. leucophaeta* to low-level chlorination (0.10–1.00 mg litre<sup>-1</sup> residual chlorine).

## MATERIALS AND METHODS

Shell valve movements of mussels exposed to five different chlorine concentrations — 0.10, 0.25, 0.50, 0.75 and 1.00 mg litre<sup>-1</sup> chlorine residuals (reached by using actual Cl<sub>2</sub> doses between 0.14 and 1.35 m litre<sup>-1</sup>) — were studied in the laboratory using a mussel monitor (Kramer *et al.*, 1989). Six to seven mussels were individually glued by means of dentists glue, (Unifast, GC Dental Industrial Corp, Tokyo) to PVC plates on the mussel monitor following procedures outlined by Jenner *et al.* (1989). Measurement of shell valve movement of individual mussels was recorded automatically every 1 min through AD-conversion (analog devices) in a PC. The relative position of the shell valves (between closed and open) was readily displayed graphically on the screen for direct observation (Jenner *et al.*, 1989).

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Experimental mussels (mean  $\pm$  SD:  $21.4 \pm 1.1$  mm shell length) were collected from the Noordzeekanaal near the Velsen power-station intake area. Mussels were initially acclimatized (48 h at  $20^\circ\text{C}$ ) in the experimental tanks under a flow rate of  $100 \text{ ml min}^{-1}$  using brackish water (mean  $\pm$  SD:  $5.1 \pm 0.1\text{‰}$  salinity;  $7.9 \pm 0.1$  pH;  $48.6 \pm 3.8 \text{ mg litre}^{-1}$  suspended particulate matter;  $33.6 \pm 5.3 \mu\text{g litre}^{-1}$  chlorophyll-*a*) collected from the Noordzeekanaal. After acclimation, the appropriate

amount of sodium hypochlorite was added using peristaltic pumps to maintain desired chlorine concentration in an experimental tank, following the procedures described by Rajagopal *et al.* (1994). Residual chlorine measurements were done using the iodometric method (White, 1972). The chlorine concentrations (residual chlorine) were determined at regular intervals in the tank to ensure a uniform distribution of chlorine residuals. The levels of residual chlorine at outlet were also monitored at 30-min intervals.

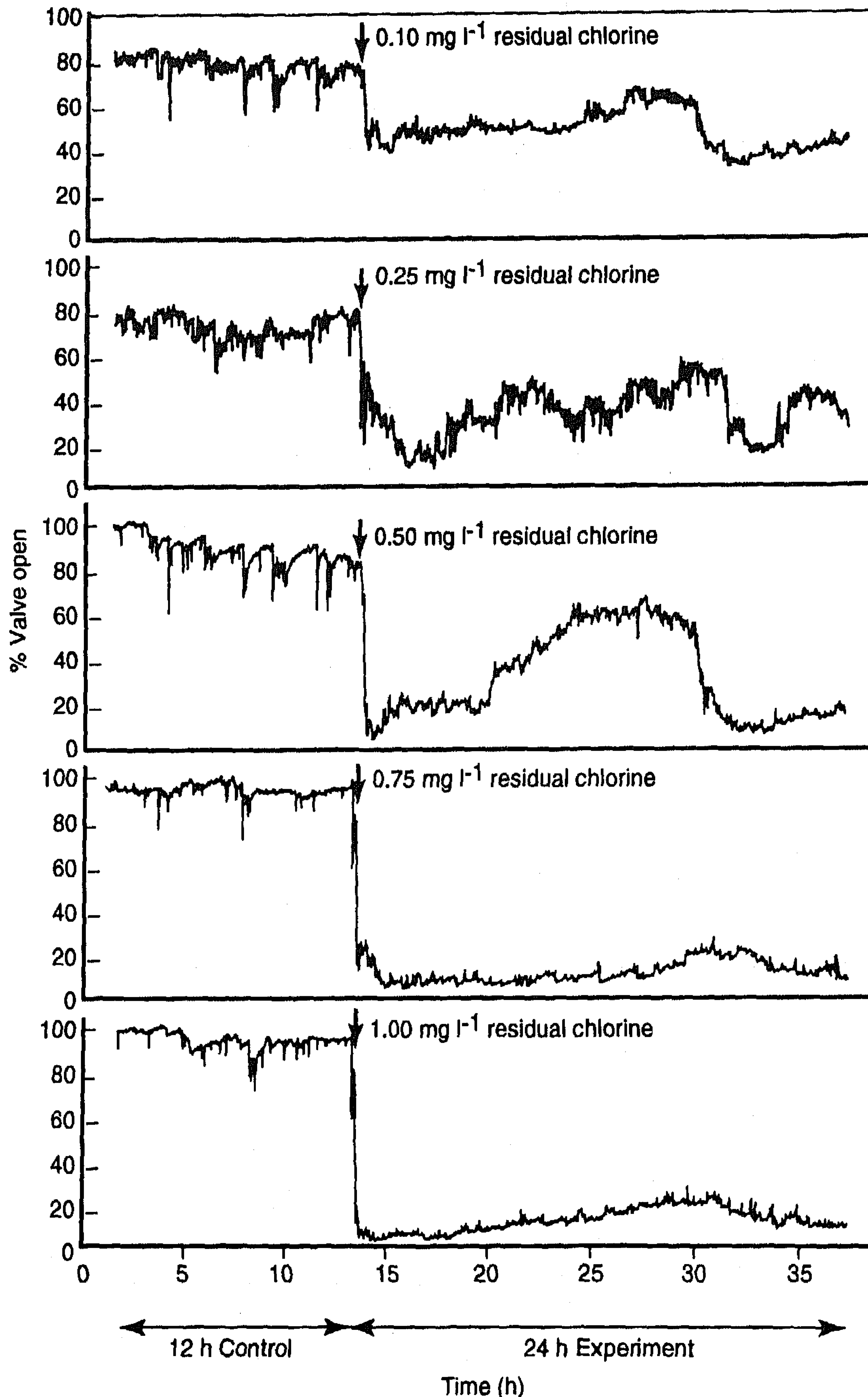


Fig. 1. Average ( $n = 6-7$ ) patterns of shell valve movements of brackish water mussel, *Mytilopsis leucophaeta*, when exposed to different chlorine residuals. The status of the shell valves was logged every 1 min. 12-h light:dark regime 07.00-19.00:19.00-07.00. Total period of experimental duration is 48 h (12 h acclimation, 12 h control and 24 h experiment).



Table 1. Statistics of shell valve movements data (% valve open) when the dark false mussel, *Mytilopsis leucophaeta* ( $n = 6-7$ ), was exposed to different doses of chlorine residuals. Total period of experimental duration is 48 h (12 h acclimation, 12 h control and 24 h experiment) for each chlorine dose. The status of the shell valves was logged every 1 min. The group-wise comparisons (control and experimental) of the shell openings at different chlorine concentrations were tested by using one-way ANOVA followed by Tukey's HSD multiple comparison test

	Total residual chlorine									
	0.10 mg litre <sup>-1</sup>		0.25 mg litre <sup>-1</sup>		0.50 mg litre <sup>-1</sup>		0.75 mg litre <sup>-1</sup>		1.00 mg litre <sup>-1</sup>	
	Control	Expt	Control	Expt	Control	Expt	Control	Expt	Control	Expt
Mean	83.51	52.15	79.94	35.70	80.11	27.73	90.74	13.48	90.04	9.36
Minimum	57.83	36.00	55.00	13.33	63.67	6.17	69.14	7.14	73.50	3.50
Maximum	91.67	69.12	86.33	61.67	100.00	62.50	100.00	32.14	100.00	36.00
Median	85.50	52.33	76.67	36.08	80.00	29.25	90.43	12.29	90.83	9.67
SD	7.42	12.22	9.07	17.10	10.30	15.81	5.99	4.07	7.33	6.45
SE	0.37	0.51	0.50	0.95	0.37	0.54	0.15	0.21	1.17	0.14
Lower 95% cl <sup>b</sup>	82.98	51.93	79.35	34.81	79.59	26.67	90.45	13.07	89.72	9.12
Upper 95% cl <sup>b</sup>	84.04	52.37	80.53	36.60	80.63	28.79	91.03	13.88	90.37	9.60
Tukey's HSD test	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$	

<sup>b</sup>Confidence limits.

Total period of experimental duration was 48 h (12 h acclimation between each chlorine dose, 12 h control and 24 h experiment) for each chlorine dose. The group-wise comparisons (control and experimental) of the shell openings at different chlorine concentrations were tested by one-way ANOVA followed by *post hoc* Tukey's honestly significant difference (HSD) multiple comparison test.

#### RESULTS AND DISCUSSION

The behavioural pattern of shell valve movements of *M. leucophaeta* to different chlorine doses and control experiments is given in Fig. 1. The percentage shell opening was found to decrease with increasing chlorine concentrations. However, the shell openings of mussels from the control experiments of 0.10, 0.25, 0.50, 0.75 and 1.00 mg litre<sup>-1</sup> were not significantly different (ANOVA,  $df = 30$ ,  $F = 2.594$ ,  $P > 0.0598$ ). At 0.10 mg litre<sup>-1</sup> residual chlorine, the shell opening of mussels was reduced to 52% from 84% (Tukey's HSD test,  $P < 0.001$ ; Table 1). Compared with shell openings at 0.10 mg litre<sup>-1</sup> residual chlorine, openings at 0.25 (36%), 0.50 (27%), 0.75 (13%) and 1.00 mg litre<sup>-1</sup> (9%) were significantly lower (Tukey's HSD test,  $P < 0.001$ ). Shell openings at 0.75 and 1.00 mg litre<sup>-1</sup> residual chlorine (Tukey's HSD test,  $P > 0.05$ ) were not significantly different. This indicates that 0.75 mg litre<sup>-1</sup> is the chlorine residual in which shell movement activities are critically affected in *M. leucophaeta*. The percentage reduction in mussel shell opening (between control and experiments) was strongly correlated (Spearman rank correlation test:  $r = 0.89$ ,  $P < 0.0001$ ) with chlorine doses.

Biofouling investigations using chlorination procedures have been concerned mainly with mussels, as they are the main macrofouling organisms in any industrial cooling system (Jenner, 1985; Lewis, 1985; Rajagopal *et al.*, 1996). The most obvious feature of a bivalve is the possession of protective shell valves. During periods of chlorination, mussels shut their valves (Khalanski and Bordet, 1980) and halt byssus production (Rajagopal *et al.*, 1994). In doing so, they isolate their body tissues from changes in the external

environment. Bayne *et al.* (1976) suggested that sensitive receptors on the mantle might be responsible for shell closure in mussels. The period they remained closed could last from 7 days (Jensen, 1982) to 35 days (Theede *et al.*, 1969), with only short and intermittent periods of shell opening (Widdows, 1973; Kramer *et al.*, 1989). Lewis (1985) noted that mussels, *Mytilus edulis* treated with chlorine residuals of 4.43 mg litre<sup>-1</sup> for 49 h, were capable of making recovery within 30 min of exposure to chlorine-free seawater. However, Jensen (1982) found that *M. edulis* failed to recover after a 24-h exposure to a chlorine residual of 8–40 mg litre<sup>-1</sup>. A review of the literature shows that at chlorine levels of  $< 1.0$  mg litre<sup>-1</sup>, mussels are able to open their valves to feed, although at a reduced rate (White, 1966). However, at higher chlorine levels, they are forced to shut their valves and exist on stored food reserves and anaerobic metabolism (Lewis, 1985) until energy resources are depleted (Jensen, 1982) or metabolic wastes (e.g. ammonia) reach a toxic level. It appears that at high chlorine residual levels (i.e. 40 mg litre<sup>-1</sup>), denaturation of cell membranes could lead to lethal effects, particularly in the gills (Opresko, 1980), but at low residuals only physiological activities are affected (Rajagopal, 1997). Therefore, experiments were conducted to ascertain the effects of chlorine administered at low levels (0.10–1.00 g litre<sup>-1</sup>) on the shell valve movements of *M. leucophaeta* which constitute a major activity associated with respiration, feeding, waste removal and byssus thread formation. The present data clearly indicated that *M. leucophaeta* was able to sense the presence of chlorine at levels as low as 0.10 mg litre<sup>-1</sup> and responded by reducing the shell opening by 31% (Fig. 1). There was a dose-dependent reduction in the shell opening up to a concentration of 1.0 mg litre<sup>-1</sup> residual chlorine. However, there was no significant difference between their response at 0.75 and 1.00 mg litre<sup>-1</sup>. Under such prolonged periods of shell closure, mussels do not get an opportunity to compensate for the energy loss incurred due to reduced food intake (Jensen, 1982),

and thereby a drastic decline of the growth rate is to be expected (Lewis, 1985).

The present study shows that the continuous dosing at a level of at least 0.75 mg litre<sup>-1</sup> is necessary to force the mussels close their shells, without allowing a recovery phase (Fig. 1). Current Dutch advisable total residual chlorine (TRC) concentration is about 0.50 mg litre<sup>-1</sup> before condenser inlet. Therefore, at least this level should be maintained during settlement periods of *M. leucophaeta* to control fresh colonization. Earlier studies have shown that peak settlement of *M. leucophaeta* is during the period of July–September in Noordzeekanaal (Rajagopal *et al.*, 1995). Following this period, the chlorine dosing may be changed to intermittent mode (0.50–1.00 mg litre<sup>-1</sup> residual chlorine measured in front of the condenser system, for 4 h in every 8 h) (Jenner *et al.*, 1996).

A survey of the available literature shows that there is hardly any data available on the response of *M. leucophaeta* to biocides (Rajagopal *et al.*, 1994). A comparison of present data with similar data for other mussel species (*Mytilus edulis* and *D. polymorpha*) indicates that *M. leucophaeta* is more tolerant to chlorine and, therefore, might present significant problems in cooling conduits of power stations.

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